been described. In KCNQ/Kv7 channels, the molecular determinants of the M-current, a slow voltage-dependent non-inactivating neuronal potassium-selective current, CaM binding is essential for channel trafficking to the plasma membrane. The goal of the present work is to investigate whether changes in the availability of CaM could influence membrane expression of Kv7.2 channels. To this end, different ratios of Kv7.2 and CaM cDNAs have been used to transfect HEK-293T cells, and the expressed current density has been assessed by whole-cell patch-clamp. When Kv7.2 and CaM were transfected at a 1:1 cDNA ratio, Kv7.2 current density was identical to that recorded in control cells. By contrast, increasing the CaM/Kv7.2 cDNA ratio to 10:1 and 20:1 caused a dose-dependent increase in current density (by 50 and 90%, respectively). Similar increases in current densities were observed when Kv7.2 cDNA was transfected at identical ratios with a mutant CaM unable to bind calcium (CaM1234). These results suggest that the amount of apo-CaM accessible to Kv7.2 is limiting for channel function, raising the intriguing possibility that changes in CaM availability meditated by binding partners (eg. neurogranin, neuromodulin), which are known to occur during long term potentation (Zhong et al., EMBO J, 28:3027, 2009), might rely on changes in M-current density.

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Kv7.2/7.3 Channels are Enhanced During Striatal Development and Promote Neuronal Functional Maturation of iPS Cell-Derived Neurons

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M-currents, encoded by Kv7.2/7.3 genes, are low-threshold, voltage-gated potassium channels which contribute to resting membrane potential (Vm) and regulate neuronal excitability. Here, we tested the hypothesis that the development of mouse striatal neurons is associated with enhanced expression and activity of M-currents and that determined whether forced expression of Kv7.2/7.3 accelerates the functional maturation of neurons differentiating from human induced pluripotent stem (iPS) cells. Neurons from the developing mouse striatum were isolated at E13, E15 and E17 and cultured in vitro for up to 14 days; functional profiles of the neurons were explored using whole-cell patch-clamp. In some experiments, subventricular and mantle zones were separated by laser microdissection and channel subunit ontogenies determined by gene chip array. Shortly after isolation (1 day in vitro), there was a clear in vivo development-dependent hyperpolarisation of Vm (E13=-24.8 ± 2.3 mV (n=26), E15=-40.0 ± 2.2 mV (n=12), E17=-47.2 ± 2.4 mV (n=9)). Although Vm continued to hyperpolarise in vitro, by 7 days these differences has disappeared (e.g. E13=-52.1 ± 2.8 mV (n=8), E17 =-54.7 ± 1.9 (n=5)). Associated with these observations, were parallel and progressive increases in M-current activity during in vivo and in vitro development, as evidenced by more pronounced responses to retigabine and XE991. Further, both Kv7.2 and 7.3 genes were progressively enriched in the mantle zone during in vivo development. Importantly, human iPS cell-derived neurons nucleofected with a concatenated Kv7.2/7.3 cDNA plasmid demonstrated a significantly hyperpolarized Vm (from −39.9 ± 2.2 mV (n=12) to −51.3 ± 1.1 mV (n=11)) and a dramatic increase in the proportion of cells generating action potentials spontaneously. Taken together, these data indicate that development of striatal neurons is associated with increased M-current expression and that this might be causative as suggested by the effect of forced Kv7.2/7.3 expression in the iPS cell-derived neurons.

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Tamoxifen Inhibition of Kv7.2/Kv7.3 Channels

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KCNQ genes encode five Kv7 K^+ channel subunits (Kv7.1-Kv7.5). Four of these (Kv7.2-Kv7.5) are expressed in the nervous system. Kv7.2 and Kv7.3 are the principal molecular components of the slow voltage-gated M-channel, which regulates neuronal excitability. In this study, we demonstrate that tamoxifen, an estrogen receptor antagonist used in the treatment of breast cancer, inhibits Kv7.2/Kv7.3 currents heterologously expressed in human embryonic kidney HEK-293 cells. Current inhibition by tamoxifen was voltage independent but concentration-dependent. The IC50 for current inhibition was 1.68 ± 0.44 μM. The voltage-dependent activation of the channel was not modified. Tamoxifen inhibited Kv7.2 homomeric channels with a higher potency (IC50 = 0.74 ± 0.16 μM). The mutation Kv7.2 R463E increases phosphatidylinositol- 4,5-bisphosphate (PIP2) - channel interaction and diminished dramatically the inhibitory effect of tamoxifen compared with that for wild type Kv7.2. Conversely, the mutation Kv7.2 R463Q, which decreases PIP2 -channel interaction, increased tamoxifen potency. Similar results were obtained on the heteromeric Kv7.2 R463Q/Kv7.3 and Kv7.2 R463E/Kv7.3 channels, compared to Kv7.2/Kv7.3 WT. Overexpression of type 2A PI(4)P5-kinase (PIP5K 2A) significantly reduced tamoxifen inhibition of Kv7.2/Kv7.3 and Kv7.2 R463Q channels. Our results suggest that tamoxifen inhibited Kv7.2/Kv7.3 channels by interfering with PIP2-channel interaction because of its documented interaction with PIP2 and the similar effect of tamoxifen on various PIP2 sensitive channels.

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KCNE1 Separates the Main Voltage Sensor Movement and Channel Opening in KCNQ1/KCNE1 Channels

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The IKs channel is a slowly activating potassium channel that generates one of the potassium currents that limits the duration of the cardiac action potential. The IKs channel consists of 4 pore forming alpha subunits (KCNQ1) and 2-4 beta subunits (KCNE1). The four KCNQ1 subunits form functional potassium channels, but the KCNE1 beta subunit is necessary to recapitulate the slow activation kinetics of the IKs channel. The mechanism by which KCNE1 slows the kinetics of KCNQ1 channels is a matter of current controversy. Here, we use a combination of voltage clamp fluorometry (VCF) and gating current measurements to show that IKs channel activation occurs in two steps: (1) mutually independent voltage sensor movements in the four KCNQ1 subunits generate the main gating charge movement and underlie the delay in the activation time course of the KCNQ1/KCNE1 currents, (2) a slower and concerted conformational change of all four voltage sensors and the gate, which opens the KCNQ1/KCNE1 channel. Gating currents develop with a similar time and voltage dependence as the first fluorescence component, as if the first component reports on the main S4 charge movement. In contrast to other Kv channels, the voltage dependences of the main voltage sensor movement and channel opening are separated by over 100 mV. The two activation steps in KCNQ1/KCNE1 channels can be farther separated by a disease-causing mutation in KCNE1. We determine rates and voltage dependence of the gating transitions to construct a model for KCNQ1/KCNE1 channels. Our model is consistent with that KCNQ1/KCNE1 channel has a fast S4 movement at negative voltages that moves the majority of gating charge and a slower second conformational change at positive voltages that moves a smaller amount of gating charge and opens the gate.

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Specific Targeting of Kv7.1/KCNE1 Channel Complexes

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The five KCNE β-subunits interact with several members of the Kv channels, thereby modifying channel gating via the interaction of their single transmembrane-spanning segment, the extracellular amino terminus, and/or the intracellular carboxy terminus with the Kv α-subunit. Best studied is the molecular basis of interactions between KCNE1 and Kv7.1, which, together, supposedly form the native cardiac I(Ks) channel. Besides biophysical effects the pharmacology of Kv7.1 is modulated by KCNE1. Kv7.1 is coexpressed with different KCNEs in tissue specific combinations. Highly selective compounds that can discriminate between Kv7.1/KCNEx-complexes may allow for tissue specific action of drug candidates. Here, we present data on the first highly potent, pure Kv7.1/KCNE1 inhibitor" that does not act on Kv7.1 alone. We determine its binding site and interpret underlying structural mechanisms of subtype selectivity.